EXPERIMENTS ON BLOOD AMYLASES

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DISSERTATION

Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in the Faculty of Pure Science of
Columbia University.

BY

MARIE MUHLFELD

NEW YORK CITY
1928

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ACKNOWLEDGMENT.

The author wishes to express her gratitude to Professor Henry C. Sherman and Dr. Mary L. Caldwell at whose suggestion this investigation was begun and under whose direction it was carried out, for their kindly interest and advice.

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EXPERIMENTS ON BLOOD AMYLASES

INTRODUCTION.

The presence of an amylolytic property in blood was recognized as early as 1846 when Magendie[1], injecting starch intravenously, found after ten minutes that the blood no longer gave a starch-iodine reaction, but that the content of blood sugar had increased considerably. Several authors confirmed these findings and there followed reports of controversy as to the seat of the active principle of the blood. It was variously attributed to the red blood cells, the leucocytes and the blood serum. Lepine and Barral in 1891 [2] and Bial [3] in 1892 were able to show that the serum contains the active principle and that the corpuscles washed free from serum were inactive to starch or glycogen solutions.

In 1909, Wohlgemuth[4, 5] proposed a more quantitative method for determination of the amylolytic value of animal fluids and published a series of papers on animal amylases.

His paper[6] on the amylase content of blood presents the following conclusions: the amylase action of serum is as strong as that of plasma and the size of the clot is without effect on the amylase; further treatment of the serum with fibrin caused no measurable effect on the amylase content; the content of amylase in the blood from the peripheral vessels is approximately the same as from the internal blood vessels; the fasting dog (even after long periods of starvation) has as much amylase in its blood as has a well fed dog; the kind of food is without effect on the amylase content of the blood. Wohlgemuth reports the smallest amounts of amylase in the blood of humans, and goats, somewhat more in rabbits and the largest amounts in guinea pigs and dogs. He expresses his findings in terms of $D^{\circ C}_{t}$, where $D^{38^\circ}_{24hr}$ is the number of cc. of one percent starch solution which can be completely broken down to the erythrodextrin stage by one cc. of the enzyme solution at a temperature of 38° C. in 24 hours. His

values are: Rabbit, $D^{38°}_{24h} = 40$; Dog, $D^{38°}_{24h} = 312.5 — 165$; Guinea pig, $D^{38°}_{24h} = 625$.

In another paper[7], incidental to his studies of the organs of normal rabbits he found the blood value to be $D^{38°}_{24h} = 62.5 — 165$ for six animals.

Many workers, interested primarily in the source of the blood and lymph amylases or in the blood sugars and the effect of drugs on these, have reported parallel studies on blood amylases. The reports of these researches are startlingly contradictory and, since different workers have used different methods, are not easily summarized. However, many have established normal blood enzyme values for their own comparisons and these figures are of particular interest.

King[8], in 1914, studied blood and urinary amylases. He carried the starch digestion to the achroodextrin stage and reported the diastatic power as D equals the cc. of one per cent starch solution carried to the achroodextrin stage in one hour. He found that the diastatic powers of the blood of ten dogs, when no attention was given to food or drink, lay between $D = 5$ and $D = 7.8$ with the average $D = 6.2$. For other species he gives the averages: cats, $D = 6.8$; rabbit, $D = 2.5$; woodchuck, $D = 18$; and raccoon, $D = 20$.

Myers and Killian[9] in 1917 reported on the increased diastatic activity of the blood in diabetes and nephritis. They determined the sugar after incubation at 40° for 15 minutes according to the technique of Myers and Bailey[10] and report their results in terms of the percentage of 10 milligrams of soluble starch transformed to reducing sugars (calculated as glucose) by 2 cc. of blood. Their normal blood diastase values for six men, in age from 25 to 29 years, vary only from 15 to 17. Thirteen cases of diabetes gave activities of 39 to 74, and twenty-three cases of nephritis ranged from normal values to 52 with eleven of the cases above 30.

Lewis and Mason[11], also interested in the blood sugar-blood amylase relationship, especially as it is affected in diabetes, practically repeated the work of Myers and Killian, using only a slightly different method[12]. They express their

results in the same units as did Myers and Killian, namely, the diastatic index is the percentage of 10 milligrams of soluble starch converted into dextrose. Of the fifty-eight determinations on normal bloods, forty-seven fell in the range 14 to 25, and showed a slight plus and minus change after meals of a single type food. Twenty-seven cases of bloods of diabetic patients, showed only six cases with a value above 15 and fourteen cases were below 10. They therefore conclude that the normal diastatic index is between 14 and 25, that the diastatic activity of the blood shows little change in relation to food and that there is no constant increase in the blood amylase in diabetes, indicating no relation between the height of blood sugar and the diastatic activity.

Using the Myers and Killian methods, Watanabe[13] studying rabbits' blood found that in spite of the hypoglycemia and glucosuria after ether anesthesia the blood amylase activity was unaffected. Also using the Myers and Killian method, S. J. Cohen[14] working on starved dogs, confirmed among other results, that whole blood and plasma show practically identical diastase activity and that red blood corpuscles are almost inactive.

Investigators using the modified Wohlgemuth method[5] have published the following normal values for human blood diastase in terms of d = cc. of 0.1 per cent starch solution digested by 1 cc. of enzyme solution in one-half hour or $d^{38^\circ}_{30'} = \frac{\text{mg. starch}}{\text{cc. enzyme}}$.

Wohlgemuth and Noguchi[15] -	8 to 16
Corbett[16] - - - - -	10
Stocks[17] - - - - -	6.6 to 8
MacLean and Wesselow[18] -	5 to 10
Harrison and Laurence[19] -	3 to 7

The disturbance of body relationships by vitamin deficiency was recognized with the recognition of the vitamins. In 1914, Funk and Schonbörn[20] reported a hyperglycemia in pigeons suffering from polyneuritis and in 1918, Dutcher[21]

showed the catalase content of the blood to be lowered 44.4 per cent in avian polyneuritis.

In 1924, Palladin[22] reported on the carbohydrate metabolism in experimental scurvy and followed the blood sugar and blood amylase values during the progress of the disease. In the twenty cases recorded in his protocols the blood sugar increased regularly until the fifteenth to the eighteenth day when it reached a maximum after which it fell rapidly, until a few days before death there was a marked hypoglycemia. Paralleling this curve of rise then fall in blood sugar, Palladin found a curve of increased then decreased amylase. His amylase curve is a composite curve of a series of determinations, the animals being killed at various time intervals of the disease. At the peak, the value reached $D^{38^\circ}_{1\ hr} = 500$, a value about 15 times his normal, and just before death, fell to $D = 4$. He feels that experimental scurvy produces marked effects on the entire carbohydrate metabolism.

Hess and Killian[23] found, however, a normal amylase content in blood of human scurvy.

Kudrjawzewa[24], in a study of the blood enzymes in experimental polyneuritis, including amylase, established a normal of $D^{38^\circ}_{24h} = 195$, the nine cases used in the average falling seven at $D = 160$ and two at $D = 320$. His polyneuritic pigeons (thirteen cases) gave an average $D = 135$, but again nine of the cases gave the value $D = 160$ and four $D = 80$. He concluded that the amylase showed a constant but unimportant reduction and was the least affected of the blood enzymes.

This condensed review of previous work upon blood amylases and especially the problem of the constancy or variations in the amylolytic activity of the blood under different conditions, shows that the evidence thus far is so confusing as to leave the whole subject obscure. Yet such knowledge is unquestionably important to a comprehensive understanding of the place of the amylolytic enzymes in nature. The present investigation does not attempt to unravel all the difficulties presented by a study of previous publications on the

subject, but seeks rather to contribute to the establishment of a better fundamental basis for further investigation and interpretation, through a somewhat intensive study of method, of individual variability, and of age and growth, in the one species now most used in laboratory studies of nutrition, namely, the rat (*Mus norvegicus albinus*).

EXPERIMENTAL

Materials. Enough starch for the investigation was prepared from a single sample of Merck's "soluble starch according to Lintner", by washing the commercial product repeatedly with distilled and triply distilled water. The starch was air dried and its moisture content determined.

All the salts used were carefully purified. Tested purity products were recrystallized three times, twice from distilled and once from triply distilled water. They were used in the air dried condition, calculated to moisture free.

Laboratory distilled water was redistilled twice, first from alkaline permanganate and then from dilute phosphoric acid. The final distillate was collected through a block tin condenser in "non-sol" bottles as required and was used for all solutions and for the final rinsing of all glassware.

The blood for the testing of amylase content was always drawn in the same way, though not always at the same time of day. However, since Wohlgemuth and Ehrmann[25] have shown that neither the length of time after feeding nor the part of the body from which the blood is drawn is a factor, it was felt that a representative sample was obtained. The rat was anesthetized with ether and after removal of the hair and interfering tissue at the neck, both the carotid artery and the jugular vein were severed. The heart then pumped the blood into a dry clean test tube, through a small funnel and the blood was allowed to clot. After some minutes the tube was centrifuged at a high speed and the supernatant fluid was used for testing.

Apparatus. The thermostat used was a Freas design, the temperature of which was controlled at $40° \pm 0.02°$ C.

The potentiometer was a Leeds and Northrup portable, recording the voltage to the fourth decimal place. Saturated calomel cells and hydrogen electrode holders were of the type described by Wilson and Kern[26]. Electrodes were of platinum, coated with platinum black and were checked against standardized tenth molar hydrochloric acid before each determination. Electrodes were used for several determinations on the same day but were never used a second day. Determinations were made at room temperature and the hydrogen ion concentration calculated by means of the equation

$$pH = \log \frac{1}{(H^+)} = \frac{\text{E.M.F. (observed)—E (calomel electrode)}}{0.0001983\ T}$$

The value $E = 0.2466$ was used throughout.

Method. Preliminary experiments showed that the gravimetric method usually employed in these laboratories for determining the saccharogenic power of amylases, could not be used in the presence of the blood proteins and amino acids because of their interference with the precipitation of cuprous oxide and the determining of reducing sugar with Fehling's solution. For this reason the amyloclastic power of blood serum was measured.

The method used is based on that of Wohlgemuth [4] and in part has been previously used and described in this laboratory[27]. Enough starch to make 500 cc. of one per cent starch paste was weighed out and transferred to a small beaker in which it was stirred to a thin paste with cold water. It was quantitatively washed into a flask containing a larger amount (to total not more than 250 cc.) of boiling water. The whole was then rapidly brought to the boiling point and boiled for three minutes. After having been cooled to room temperature the contents of the flask were carefully washed into a volumetric flask containing the proper amount of buffered salt solution and brought to volume.

For the 0.25 per cent starch paste used for the half hour tests, 50 cc. of the one per cent solution were brought to 200

cc. in a calibrated volumetric flask with the properly prepared salt solution. The pH of the starch solutions was tested and maintained at 7.0-7.1 and the final concentration of salt was in each case: 0.05 M. sodium chloride; 0.005 M. disodium phosphate; and enough dihydrogen phosphate to maintain the desired hydrogen ion activity.

The blood serum was diluted 1:100 with a solution of the same concentrations of the same salts as the starch and at the same pH. Whenever possible (in almost all cases except when sufficient blood was not obtained) two tests were carried out on each blood, one duplicating Wohlgemuth's procedure in which the enzyme acted on 5 cc. of a one per cent starch paste (50 mg. starch) for 24 hours, and another in which the enzyme acted on 2.5 cc. of a one-quarter per cent starch paste (6.25 mg. starch) for thirty minutes.

A series of clean dry cold test tubes was prepared with increasing amounts of the diluted blood serum. This was carefully introduced into the bottom of the test tubes by means of calibrated pipettes. For the smaller amounts used in the 24 hours series, a one cc. pipette graduated to 0.01 cc. and bearing a Bureau of Standards certificate was used. For the larger amounts a calibrated 5 cc. pipette graduated to 0.05 cc. and likewise bearing the Bureau certificate was used. Each tube was then brought to a volume of 5 cc. with a solution containing 0.05 M. sodium chloride and 0.005 M. phosphate adjusted to $pH = 7$ and to each was added the required amount of starch. This was done very carefully by means of a long tipped burette in order to avoid the lodging of starch on the side walls of the tube. The tube, when thus prepared, was immediately placed in a wire basket in ice water until the entire series was ready.

The basket of tubes all with the same total volume, salt content and amount of starch, and with increasing amounts of blood serum, was placed in the thermostat and at the expiration of the experimental period, thirty minutes, four, or twenty-four hours, was taken out and placed in the ice bath, to stop the enzymic action. Thus the tubes were all subjected to

an average temperature of 40° C. for the time period. After a few minutes on ice, 0.1 cc. of 0.1 N iodine in potassium iodide solution was added. Since variation in the concentration of iodine will give serious differences in color value, the addition of iodine must be carefully controlled. In these tests a dropping bottle which delivered drops of 0.1 cc. volume was used. Each series was then observed for the end point, that is, the tube which shows a clear red color as compared with the Milton Bradley color charts and has no trace of the blue or violet color due to starch.

Table I gives a typical example of the records obtained with the serum of a normal animal.

TABLE I.

Typical Record Obtained Upon the Serum of a Normal Rat.
(Rat No. 16012 F^{RR}. Weight 192 gm. Age 17 months. Diet 148*)

Series A. Time: 30 minutes. Starch 6.25 mg. per tube (2½ cc. of 0.25% starch solution)			Series B. Time: 24 hours. Starch 50 mg. per tube (5 cc. of 1% starch solution)		
Serum Vol. used of diluted 1 : 100 cc.	Equiv. to undiluted cc.	Color	Serum Vol. used of diluted 1 : 100 cc.	Equiv. to undiluted cc.	Color
1.	0.01	B	0.3	0.003	B
1.5	.015	B	.4	.004	BV
2.	.02	B	.5	.005	V
2.5	.025	VB	.6	.006	V
3.	.03	V	.7	.007	RV
3.5	.035	RV	.75	.0075	RV
4.	.04	VR	.8	.008	VR
4.5	.045	VR(pale)	.85	.0085	VR
5.	.05	R†	.9	.009	VR
5.5	.055	OR	.95	.0095	VRRR
6.	.06	RO	1.0	.010	R†
6.5	.065	O	1.2	.012	O

* Diet 148 = Adequate diet of whole milk and wheat.
† A clear red color which marks the end point.

RESULTS

Development of Method. Wohlgemuth early realized the activating effect of salt [28] and thereafter used sodium chloride in all his determinations. The necessity of controlling the hydrogen-ion activity of the system has also been recorded but almost no work upon the amylase content of tissues or blood has been carried out under such control. Cole[29] in his text emphasizes the need of a buffer but in his directions for measuring enzyme activities makes no mention of such. Sladden[30], in 1922, in a study of the amylase of urine adjusted the pH of the urine to 6.7 (by titrating to faint pink of phenol red with either 0.10 M sodium hydroxide or 0.10 M hydrochloric acid). He says that buffering with phosphate would be advantageous but that the presence of phosphate buffer tends to obscure the reading of final results. He therefore omits the buffer but continues to adjust his original enzyme solution to pH 6.7. Harrison and Lawrence[19] paid no attention to hydrogen ion activity but in a note say that when they used buffered solutions their values were increased as much as twenty per cent.

In view of these results a few preliminary measurements were carried out in which the amylase activity of the blood buffered at pH 6.9 with solutions containing either 0.0005 M or 0.005 M disodium phosphate and enough dihydrogen phosphate to bring the solution to a pH of 6.9 were studied. In all cases the better buffered solutions gave higher activities and we therefore used the higher concentration in all our studies.

As has been pointed out above, Wohlgemuth carried out his measurements of the amylase content of various tissues and fluids by using relatively large quantities of the "enzyme" solution and relatively large weights of starch, 50 mg., during an experimental period of 24 hours. In the previous work in this laboratory this technique has been modified by shortening the experimental period to 30 minutes with a corresponding decrease in the amount of starch and enzyme required. In view of the amount of work which Wohlgemuth had carried

out along this line it seemed of interest to make our measurements as far as was feasible in a manner which would make it possible to compare our results directly with his. It was decided therefore to make our measurements whenever enough blood was available in duplicate, using 50 mg. of starch and a 24 hour period and also 6.25 mg. of starch and a 30 minute period. In addition as a further check when sufficient material was available, another series of measurements using 50 mg. starch and a 4 hour period was also carried out.

As is shown in Table II the coefficient of variability was about the same for all three periods.

TABLE II.

A Comparison and Statistical Treatment of the Data Obtained in Measuring the Amylase Activity of the Serum of Some Normal Rats

Rat No.	50 mg. Starch Time = 24 hrs. cc. Serum	50 mg. Starch Time = 4 hrs. cc. Serum	6.25 mg. Starch Time = 30 min. cc. Serum
17593	0.006	0.03	0.03
17416	.007	.04	.04
17316	.007	.04	.03
17417	.0075	.05	.04
17599	.008	.05	.045
17380	.006	.035	.03
17652	.007	.045	.035
17653	.0075	.05	.04
17655	.007	.04	.033
17654	.008	.045	.037
17626	.0075	.045	.04
17629	.006	.035	.03
17405	.0075	.04	.04
17408	.009	.06	.045
17445	.010	.06	.05
17410	.009	.045	.045
17193	.007	.04	.03
17194	.008	.045	.03
Average	.0075	.0442	.0372
Standard Deviation	.00104	.0077	.0063
Coefficient of variability	14	17	17

A comparison of the results obtained in the different periods may be of interest. The amylase activity can best be compared in terms of the milligrams of starch which one cc. of serum converts to erythrodextrin. In a typical case these values expressing the amylocastic powers of the enzyme were found to be $d^{40°}_{30'} = 168$ for the half-hour period and $d^{40°}_{4h} = 1131$ for the four hour series, or it was found that in eight times the time the enzyme digested 6.7 times as much starch. The 24 hour period resulted in $d^{40°}_{24h} = 6666$, a value 5.9 times that obtained for the four hour and 39.6 times that for the half hour.

Blood Amylase in Normal Young Adults. The first experiments were carried out with normal healthy rats six months to a year of age. These animals had been raised on a diet made up of one-third whole milk and two-thirds whole wheat and sodium chloride. This diet has been shown in this laboratory to be adequate for the normal nutrition of the rats through several generations. For nineteen of these normal animals the amylase value of the blood serum given as the average number of cc. necessary to digest 6.25 mg. of starch to a point at which it would give a clear red color with iodine was 0.033. In terms of $d^{40°}_{30'} = \frac{\text{mg. starch}}{\text{mg. enzyme}}$ the value is 190. When 24 hour periods were used and 50 mg. instead of 6.25 mg. of starch were converted to erythrodextrin the blood serum of the same rats gave values of 0.0066 cc. or a $d^{40°}_{24h} = 7575$. (Table III). Further analysis of these results showed no significant difference in the blood of male or female animals.

TABLE III.

Data for Normal Rats

Rat No.	Sex	50 mg. Starch Time = 24 hr. cc. serum	6.25 mg. Starch Time = 30 min. cc. serum
17593	M	0.006	0.03
17531	M	.008	.04
17412	M	.007	.035
17416	F	.007	.03
17316	M	.007	.03

TABLE III (Continued)

Rat No.	Sex	50 mg. Starch Time = 24 hr. cc. serum	6.25 mg. Starch Time = 30 min. cc. serum
17417	F	.0075	.04
17599	F	.008	.045
17380	F	.006	.03
17185	F	.005	.025
17155	F	.006	.03
17154	F	.0075	.04
16824	F	.007	.03
16966	F	.005	.03
16984	F	.008	.05
17379	M	.006	.03
17393	F	.005	.025
17600	F	.007	.035
17381	F	.0055	.025
17157	F	.006	.033
Average		.0066	.033
Probable error of mean		.00015	.00103
Coefficient of variability		15.1	20.6
		$d\ ^{40\,0}_{24h} = 7575$	$d\ ^{40\,0}_{30'} = 190$

Blood Amylase Independent of Considerable Variation in Vitamin A Intake. At the same time that these normal animals were being studied, there were being tested in the laboratories two diets of graded vitamin A content. Diet 77 contained approximately one-half and Diet 78 one-quarter the vitamin A content of Diet B. These diets were thus decidedly lower in vitamin A than the diet used to establish the normal, yet were not so deficient as to throw the animals into a pathological condition. Though representing just about the lower boundary of the zone of normal nutrition so far as vitamin A is concerned, the weight and breeding records of the rats were not materially affected. Table IV gives the data of the experiments with these rats and it will be seen that there was very little difference in the average amylase content of the animals on these diets, both low in vitamin A.

TABLE IV.

A Comparison of the Amylase Content of the Blood Serum of Rats on two Diets both Low in Vitamin A.

Rats from Diet 77				Rats from Diet 78			
Rat No.	Sex	50 mg. Starch Time = 24 hrs. cc. Serum	6.25 mg. Starch Time = 30 min. cc. Serum	Rat No.	Sex	50 mg. Starch Time = 24 hrs. cc. Serum	6.25 mg. Starch Time = 30 min. cc. Serum
18235	F	0.0065	0.03	18222	M	0.007	0.03
17404	M	.0085	.04	18226	F	.005	.03
17405	M	.0075	.04	18227	F	.009	.045
17408	F	.009	.045	17652	M	.007	.035
17445	F	.010	.05	17653	M	.0075	.04
17410	F	.009	.045	17655	M	.007	.033
17193	M	.007	.03	17654	M	.008	.037
17194	M	.008	.03	17626	M	.0075	.04
17197	F	.008	.045	17629	M	.006	.03
17196	M	...	.04	17658	F	.007	.035
17198	F	...	.035	17659	F	.005	.025
17561	F	...	.048	17627	M	.007	.035
17407	F	.008	.045	17166	F	.0085	.045
17409	M	.0085	.045	17235	F	.0085	.04
17406	M	.004	.02				
Average		.00783	.0392	Average		.00714	.0357
Probable error of mean			.00135	Probable error of mean			.00101
Coefficient of variability			20.6	Coefficient of variability			15.9

$d\,\frac{40^{\circ}}{24h} = 6400$ $d\,\frac{40^{\circ}}{30'} = 160$ $d\,\frac{40^{\circ}}{24h} = 7000$ $d\,\frac{40^{\circ}}{30'} = 175$

Difference between means = .0035
Probable error of difference = .00168

When the bloods of rats raised on Diets 77 and 78, low but not deficient diets, are related to the normal of the same group, we find again that the differences between the averages are not significant. Comparing the amylase activity of the serum of rats raised on Diet 77 in terms of the cc. of serum required to bring the starch to the end point, it was found that the difference between the means is 0.0062 (Diet 77, 0.0392 — Diet B, 0.033) and that the probable error of this difference is 0.00172. The same comparison between rats from the normal diet and the still poorer Diet 78, shows a difference of

0.0027 (Diet 78, 0.0357—Diet B, 0.033) with a probable error of 0.00146. As these differences do not appear to be significant these diets may be considered as not affecting the blood amylase and these data confirm the findings of Wohlgemuth[6], of Watanabe[13], and of Lewis and Mason[11], that variations of adequate diet have very little effect on the blood amylase.

Blood Amylase in Animals Stunted by Diet Deficient in Vitamin A. Having determined that the blood amylase for young rats is constant despite considerable variations in vitamin A content of the diet, our next problem was to study a more drastic deficiency. As is the custom in studying vitamin

TABLE V.

A Study of the Amylase Content of the Blood Serum of Rats Stunted by Vitamin A Deficiency

Rat No.	Sex	Weight	Eight Weeks Gain	cc. Serum
18546	F	56	— 13	0.004
18542	F	63	— 8	.0075
18564	M	73	— 3	.005
18668	F	66	— 1	.006
18545	F	70	+ 1	.004
18540	M	87	+ 5	.002
18509	F	92	+ 8	.0045
18380	M	101	+ 19	.0075
18508	F	119	+ 23	.011
18599	M	135	+ 23	.011
18598	M	112	+ 25	.005
18515	M	115	+ 26	.009
18602	F	125	+ 27	.012
18516	M	121	+ 31	.010
18603	F	114	+ 32	.0065
18517	F	116	+ 32	.015
18600	M	148	+ 33	.015
18518	F	106	+ 39	.011
18507	F	127	+ 42	.010
18505	M	118	+ 48	.011
Average				.00835
Probable error of mean				.00054
Coefficient of variability				43

$$d\,{}^{40\,0}_{24h} = 6000$$

A, the rats are kept until they have reached constant weight on a diet entirely free from the vitamin, though adequate in all other respects. At this point the food to be tested is added to the diet and the experiment is continued eight weeks. The potency of the food as a source of vitamin A is indicated by the net gain in weight of the animal during the eight weeks experimental period.

We studied any available rats which had survived the experimental period. These rats varied little in age but greatly in weight, though none approached its normal weight. Since some of the rats were very small and little blood was obtained, only twenty-four hour measurements were made. The results were extremely variable suggesting that individual variability was increased when the animals were thus "attempting to grow" under the relatively restricted intake of vitamin A. Those rats which had made no gain or very slight gain had more active amylase than those which had made better gains and than normals.

The results arranged in order of increasing net gains of body weight are shown in Table V.

While the average concentration of blood amylase was not greatly different from that found in normal young rats as shown in Table III above, its coefficient of variation was much greater among the rats which had been more or less drastically stunted in growth by shortage of vitamin A, that is 43 for these stunted rats as compared with 15 for the normals.

Amylase in Relation to Age. Another problem of interest, in view of the decreased basal energy metabolism in old age in man, was the blood amylase of old as compared with young animals. Is the reduced metabolic rate a function of, or in any way correlated with, decreased enzyme activity? That the comparison might be a true one and age be the one variable, only normal rats raised on Diet B were used. Young rats, six to eight months old, were compared with rats eighteen to twenty-four months old. The first series contained eight rats each and gave the following averages: young 0.035

cc. and old 0.046 cc. (Table VI). The difference between these averages is eight times the probable error of the difference, and such a difference should be a true one.

In attempting to confirm these data with increased numbers of cases, another series of eight rats at each age was studied. The amylase measurements were carried out during and immediately after a period of extremely hot weather and the amylolytic activity of all the bloods was markedly lowered. (Table VI). The old rats again showed, however, much lower values than the younger rats.

TABLE VI.

The Amylase Content of the Blood Serum of Young vs. Old Rats All Raised on an Adequate Diet, Diet B.

YOUNG RATS			OLD RATS		
Rat No.	Sex	cc. Serum	Rat No.	Sex	cc. Serum
17593	M	0.03	15020	F	0.045
17531	M	.04	15485	F	.05
17412	M	.035	16012	F	.05
17416	F	.03	16011	F	.045
17316	M	.03	15017	M	.045
17417	F	.04	14388	F	.04
17599	F	.045	14389	F	.05
17350	F	.03	13650	F	.045
Average		0.035	Average		0.0462
Probable error		.00124	Probable error		.0007
18369	M	0.035	15926	F	0.06
18200	M	.045	15927	F	.055
18204	F	.05	15877	F	.055
18256	F	.025	15874	F	.035
18318	F	.045	15873	F	.055
18317	M	.045	16215	F	.05
18368	M	.05	16216	F	.04
18457	F	.045	15875	F	.05
Average		0.0425	Average		0.050
Probable error		.0018	Probable error		.0011
16 cases Young		.03875	16 cases Old		.0481
Probable error		.0013	Probable error		.00104

$d \frac{40}{30'} = 160$ $d \frac{40}{30'} = 130$

Since in each series the number of young and old rats was the same, the totals for the sixteen rats at each age are directly comparable. In spite of the relatively large variations caused by the discrepancy between normal and the lower values due to the increased temperature, the difference between young and old is 5.6 times the probable error.

To confirm this difference in the blood amylase activity of old and young rats, we computed the values for all the young rats between six and eight months and all our old rats without regard to small differences of diet, so long as the diet was adequate, and excluding those examined when the temperature was abnormally high. These data represent thirty-one cases of young and eighteen cases of old rats. The young rats give an average of 0.0366 cc. as compared with an average of 0.0475 cc. for old rats. The difference between the means is 0.0109 or five and one-half times the probable error of the difference, 0.00196. Since in all our averages the blood of older rats showed less amylolytic activity and the difference in each case was greater than 5.5 times the probable error of the difference, we feel the difference to be a true one and may by future research be useful in explaining the role of the amylases in nature.

SUMMARY.

Previous investigations of the amylolytic activity of blood and its possible variability under different conditions of nutrition, had yielded results which were not conclusive as to whether nutritive conditions do greatly influence the concentration of blood amylase, or whether the reported differences were chiefly attributable to differences (or possibly inherent inadequacies) in the methods used for measuring the amylolytic activity of the blood or blood serum.

The first part of the present investigation consisted, therefore, in the development of a method for the quantitative determination of blood amylase (or of the amylolytic activity of blood serum) under properly controlled conditions in the light of much recent research in this laboratory upon amylase action. The details of this method as worked out have been given.

The results are expressed both directly in terms of the cc. of blood serum required to give the defined end point, a stage at which a clear red color with iodine is obtained and in terms of $d^{\circ C.}_{t} = \frac{\text{mg. starch}}{\text{cc. blood serum}}$.

On account of their suitability to this work and the fact that they are so widely employed in studies upon varied problems in the chemistry of food and nutrition, rats were selected as the experimental animals for the investigation of the extent of individual variations in the amylase content (amylolytic activity) of normal blood, and the possible relationship of this to nutritive conditions and age.

The blood amylase of rats of comparable age which had always received diet known by long experience in this laboratory to be fully adequate for all phases of nutrition, was found to be fairly constant, the average amount of serum required to digest 6.25 mg. of starch being 0.033 cc., the maximum variations from the mean being + 0.012 to — 0.008, and the coefficient of variation in a series of nineteen rats being 20.

The blood of rats of comparable age receiving diets much lower in vitamin A than those just mentioned, but not so re-

stricted as to interfere with the normal growth and health of the animals, was found to fall within the same range of amylase content or amylolytic activity. Thus the blood amylase appears to be independent of the vitamin A intake throughout what may be regarded as approximately its normal range.

Severe deficiencies of vitamin A intake, such as to cause marked stunting or in some cases even loss of weight of young rats, appeared to increase the individual variability of the animals in blood amylase (amylolytic activity of blood serum).

Rats at about the age at which the first signs of senility usually begin to appear showed a lower concentration of blood amylase than young rats. This was found by averaging data collected over a period of about ten months covering the autumn, winter, spring and early summer. An intensive series of comparisons carried out during hot weather in midsummer indicated that under such weather conditions the amylolytic activity of the blood of both young and old rats was somewhat diminished; but fully confirmed the finding that the blood of young rats has a higher amylase content (amylolytic activity) than that of old rats of the same inheritance and environment. Thus the results of this investigation seem definitely to establish a lowering of blood amylase concentration or activity with the incipient onset of old age in the rat.

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H. C. Sherman and Marie Muhlfeld, Growth and Reproduction upon Simplified Food Supply II. Jour. Biol. Chem. **53,** 41 (1922).

P. A. Levene and Marie Muhlfeld, On the Identity or Non-Identity of Antineuritic and Water Soluble Vitamins. Jour. Biol. Chem. **57,** 341 (1923).

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Poughkeepsie, N. Y.